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Innate immune system gene polymorphisms in maternal and child genotype and risk of preterm delivery

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Abstract

Objective—There is little information about the combination of genetic variability in pregnant women and their children in relation to the risk of preterm delivery (PTD). In a sub-cohort of 487 non-Hispanic white and 288 African-American mother/child pairs, the Pregnancy Outcomes and Community Health Study assessed ten functional polymorphisms in nine genes involved in innate immune function.

Methods—Race-stratified weighted logistic regression models were used to calculate odds ratios for genotype and PTD/PTD subtypes. Polymorphisms significantly associated with PTD/PTD subtypes were tested for mother/child genotype interactions.

Results—Three maternal polymorphisms (IL-1 receptor antagonist intron two repeat (IL-1RN), matrix metalloproteinase-9 -C1562T, and TNF receptor two T198G (TNFR2)) and three child polymorphisms (IL1-RN, tumor necrosis factor-alpha -G308A, and TNFR2) were associated with PTD, but associations varied by PTD subtype and race. Two interactions were detected for maternal and child genotype. Among non-Hispanic white women, the odds of PTD was higher when both mother and child carried the IL-1RN allele two (additive interaction $p<.05$). Among

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African-American women, the odds of PTD was higher when both mother and child carried the TNFR2 G allele (multiplicative interaction p<.05).

Conclusion—These results highlight the importance of assessing both maternal and child genotype in relation to PTD risk.

Keywords

premature birth; inflammation; maternal & child genotype; gene-gene interactions; genetic polymorphisms; premature birth genetics

Introduction

Preterm delivery (PTD) is a leading cause of infant mortality and a major contributor to child neurodevelopmental problems. The U.S. PTD rate has risen 30% since 1981 with 12.7% of all pregnancies in 2005 delivered preterm [1]. The causes of PTD are not fully understood but multiple, complex, and sometimes overlapping pathways appear to be involved. Several findings suggest that inflammatory processes contribute to the etiology of PTD. For example, high levels of pro-inflammatory cytokines collected in mid-pregnancy maternal serum, mid-pregnancy amniotic fluid, and mid-pregnancy cervical samples have been associated with an increased risk of spontaneous PTD; although these findings have not been consistent across all studies [2-4]. Activation of inflammatory pathways and cytokine production can induce prostaglandin synthesis and production of collagenases. In turn these substances may initiate uterine contractions and remodeling of the collagen in the cervix and gestational membranes which ultimately culminates in preterm labor and/or preterm rupture of membranes [5].

Variability in the genome of pregnant women affects susceptibility to exogenous and endogenous factors and these interactions might trigger pathways culminating in PTD. For example, one study reported that maternal genomic variations influence inteleukin-1β response to anaerobic Gram-negative bacteria and *Gardnerella vaginalis* in the vagina and the risk of spontaneous PTD [6]. Variability in fetal genome also influences local immune responses to environmental factors that may alter the risk of PTD and sequelae of prematurity [7]. Findings from twins studies estimate the heritability of PTD to be about one-third [8], with data from large epidemiologic studies suggesting that maternal genotype contributes more substantially than paternal genotype to the risk of PTD [9]. Although the effect of maternal or child genotype has frequently been analyzed in separate studies, it has less often been analyzed in the same study, and rarely have interactions between maternal and child genotype been tested. Most recently the identification of a maternal/child genotype interaction in a large case-control study of over 700 polymorphisms lends support to the hypothesis that maternal and fetal genotype may interact to increase the risk of PTD [10].

In the Pregnancy Outcomes and Community Health (POUCH) Study, we studied ten genetic polymorphisms in mothers and their children. The polymorphisms were selected on the basis of published associations with PTD [11-15] and associations with variation in the extent of inflammation processes (see Table II) [7,12,16-21]. We choose to analyze polymorphisms in candidate genes rather than explore genome-wide associations. The candidate gene approach

has the advantage of maximizing inferences about biologic plausibility and disease causality when previous information exists about the function of the gene in disease processes, however, this approach is not ideal for initial gene discovery. One of our main objectives was to evaluate the interaction between maternal and child genomic variations in relation to the risk of PTD using the candidate gene approach.

Methods

Study Population and Data Collection

The POUCH Study enrolled a cohort (n=3,038) of pregnant women at 15-27 weeks gestation from 52 participating clinics in five Michigan communities from 1998 through 2004. The study was designed to assess biologic and psychosocial risk factors for PTD. Detailed methods have been given elsewhere [22]. Women were sampled at the time of prenatal maternal serum alpha-fetoprotein (MSAFP) screening (15-22 weeks). Women with unexplained high MSAFP (i.e. > 2 multiples of the median in singletons with no birth defect) were over-sampled (7% of cohort) due to a special interest in this biomarker in relation to PTD. Exclusion criteria included known fetal anomalies, preexisting diabetes, multi-fetal gestations, and non-English speaking. POUCH Study participants were found to be comparable on most maternal characteristics when compared to women delivering in the respective communities. At enrollment, participants provided biologic samples and completed both a self-recorded questionnaire and an in-person interview that included demographic data. A woman who indicated that she belonged to more than one racial group was asked to select the one that best described her racial identity. In this analysis, the child was considered to be of the same race as the mother. Analyses presented here were conducted in a sub-cohort (n=1371) that was sampled for in-depth investigations. The sub-cohort was randomly sampled at enrollment based on a stratum-specific sampling probability (varied by race/ethnicity and MSAFP level). At delivery the final sub-cohort was defined and included all pre-sampled cohort women and all cases of PTD. The final sub-cohort included all women who delivered preterm, those with unexplained high MSAFP, and a race-stratified random sample of women who delivered at term and had normal MSAFP. The clinical data were obtained in the sub-cohort through medical chart review by study nurses and physicians.

Definition of preterm delivery

Gestational age at delivery was estimated by using the date of last menstrual period (LMP). Dating was based on the earliest ultrasound if the ultrasound-based estimate differed from the LMP-derived estimate by more than two weeks. PTD was defined as delivery before the 37^{th} week of gestation. In some analyses, the results were stratified according to the clinical circumstances of the PTD. Delivery that occurred by Cesarean section or induction without prior spontaneous labor or rupture of membranes were grouped into medically indicated (MI) births. PTD cases in which the onset of labor or rupture of membranes occurred as the initiating event were considered spontaneous (SPT).

Child DNA collection

Between 2005 and 2007, DNA was collected from children born to women in the POUCH Study subcohort. Women whose fetus/child had died (n= 19), those who had declined to participate in any future studies at enrollment in the cohort (n=69), or who were deceased (n=3) could not be included in this follow-up study. Attempts to contact the remaining sub-cohort women (n=1280) were made via mail and telephone. During the study follow-up period 1,024 sub-cohort women were located and 1,006 were contacted. Ten women were no longer living with their child and were not able to give consent for their child to participate. Forty-one mothers who were contacted during the follow-up period declined to participate in the child DNA collection. Child DNA collection kits were mailed to 955 consenting women and 865 kits were returned (68% of the 1280 eligible). The collection kit contained ten sterile cotton swabs to collect buccal cells from inside the child's mouth. The swabs were placed into a tube containing a sodium dodecyl sulfate-based buffer and shipped overnight back to the study [12]. Fifty mother/child pairs belonged to race/ethnic groups other than non-Hispanic white and African American. This number was too small for a meaningful analysis and therefore these pairs were excluded. In 36 mothers DNA was unavailable or unsuitable for testing. In 4 mother/child pairs genotypes that were incompatible with maternity were found across multiple assays (homozygous rare genotype with homozygous common genotype pairings) suggesting an error in sample collection or handling. These samples were excluded from analyses leaving 775 mother/child pairs for final analysis.

DNA Extraction and Genotyping

Maternal genomic DNA was extracted from venous samples collected at enrollment using the Gentra Systems (Minneapolis, MN) Puregene™ kit. DNA of the children was extracted using a phenol/chloroform method from buccal swabs (Puritan Cotton Tipped Applicators REF 803-PC) collected by their mothers [23].

For maternal TNF-α and IL-1β polymorphisms, genotyping was performed using TaqMan® Assays-on-Demand™, which consist of polymerase chain reaction (PCR) primers and a fluorescently-labeled probe (Applied Biosystems, Foster City, CA). Two primer/probe sets were used that were specific to SNPs in IL-1β or TNF-α. End-point fluorescent detection was performed on an ABI Prism® 7900HT Sequence Detection System. Genotypes were evaluated using SDS software (v.2.1). Duplicate analyses were performed on a randomly selected sample representing ≥ 15% of the individuals and a reproducibility rate of 99.9% was observed.

The remaining eight maternal and all child polymorphisms were genotyped using PCR and primer pairs that spanned the polymorphic regions followed by restriction enzyme digestion and agarose gel electrophoresis according to previously published protocols: rs# 73271540 in CD14 [11], rs# 4986790 in toll-like receptor-4 (TLR-4) [16], a variable number of tandem repeats in intron 2 of interleukin-1 receptor antagonist (IL-1RN) [13], rs# 1143634 in interleukin-1 beta (IL-1β) [13], rs# 1800629 in tumor necrosis factor alpha (TNF-α) [24], rs# 72863489 in tumor necrosis factor receptor 2 (TNFR2) [25], rs# 978522 in Fas (TNFSR6) [26], rs# 73622645 in matrix metalloproteinase 9 (MMP-9) [27], rs# 1800450 and rs#

1800451 in mannose binding lectin (MBL) [28,29]. Incompatible maternal/child genotype combinations were found for 39 polymorphism assay results. These discordant results were not clustered within any single mother/child pair, therefore we assumed the errors occurred within each assay process; 39 errors with 15,500 assays is equivalent to an error rate of 2.5%. We excluded these inconsistent mother/child results from individual analyses but did not exclude the mother/child pair from the entire dataset.

Statistical Analysis

The analytic plan involved evaluation of the ten candidate polymorphisms in relation to risk of PTD using race-specific models, first considering maternal genotypes and then considering child genotypes. To reduce type I errors we did not consider all possible mother/child gene interactions. We focused on positive polymorphisms from the aforementioned analyses and considered potential maternal and child genotype interaction within a single gene. All models were stratified on race to rule out potential effects due to population stratification and because of the higher rate of inflammation/infection-related PTD among African Americans compared to that in non-Hispanic whites [30].

For descriptive purposes, Chi-square statistics were used to: 1) compare maternal characteristics in POUCH Study mothers who participated in the child DNA collection to maternal characteristics in the entire POUCH Study sub-cohort; 2) test for Hardy Weinberg equilibrium among each polymorphism in race-stratified groups of women who delivered at term with normal MSAFP; and 3) compare the race-specific allele frequencies among women who participated in the child DNA collection and women who did not have a child DNA sample.

Genotypes were divided into two classes with the homozygous common allele genotype as the reference group and the heterozygotes and the homozygous rare-allele genotypes grouped as the ‘risk’ group, also known as the dominant model. This strategy was chosen to maximize statistical power and to limit the number of comparisons. IL1-RN had more than two allelic forms, but alleles three, four, and five were very rare and were excluded (n=18). Based on previously published data, allele two was treated as the ‘risk’ allele and the allele one homozygotes were used as the reference group [6].

We applied weighted logistic regression (SAS Version 9.1.3 Proc Surveylogistic) to examine potential differences in the odds of PTD associated with maternal and child genotypes and their interactions. All regression analyses incorporated sampling weights accounting for oversampling of high MSAFP, African-American women, and PTD.

In weighted logistic regression, we considered maternal genotypes first and then child genotypes. Both maternal and child genotypes were then considered for potential genotype interaction within a single gene. The effect of genotype interaction on PTD risk was tested for the four genetic polymorphisms that had significant maternal or child genotype main effects on PTD: IL1-RN (non-Hispanic white), IL-1β (African American) MMP-9 (non-Hispanic white), TNF-α (African American) and TNFR2 (African American). The significance of interactions was tested on the multiplicative (p-value for the interaction term from logistic regression) and additive scales (relative excess risk due to interaction) [31].

The response categories in the weighted logistic regression were first binary (i.e. Term or PTD) and then polytomous (i.e. Term (Ref), MI PTD, or SPT PTD). The modeling of clinical subtypes of PTD was planned a priori because distributions of causal pathways differ across these subtypes. However, in the analyses of the maternal child genotype combinations we used only the binary outcome due to limited power.

Analyses were repeated using Cox proportional hazards models adapted to the case-cohort design (Proc PHREG) to calculate hazard ratios and confidence intervals with standard errors based on the robust sandwich estimate [32]. The risk estimates and odds ratios obtained from logistic models and the proportional hazard models were similar and the findings related to the combinations of maternal and child genotype did not change. Here we present results from the logistic models only.

Results

There were 487 non-Hispanic white (n=131 PTD) and 288 African-American (n=49 PTD) maternal child pairs in this sample (Table I). The majority of non-Hispanic whites (66%) and a minority of African-American women (36%) had greater than a high school education. For both African Americans and non-Hispanic whites ∼40% of women were primiparous. The PTD rate was 10% in non-Hispanic white women, and 14%, in African-American women. In race stratified comparisons, characteristics (maternal age, maternal education, Medicaid Insurance use, parity, and PTD rates) of women whose children had genetic analysis did not differ significantly ($p > .05$) from those in the entire POUCH Study sub-cohort (data not shown).

The MMP-9 polymorphism in the non-Hispanic white women was the only genotype that deviated from Hardy-Weinberg equilibrium. Race-specific polymorphism frequencies among women who delivered at term and preterm are presented in Table II. Maternal allele frequencies did not differ between the sample with child DNA (participants in the follow-up collection) and the sample without child DNA (non-participants in the follow-up collection) (data not shown).

Results from analysis of maternal polymorphisms in relation to PTD are presented in Table III. In non-Hispanic white women significant findings included the IL1-RN allele 2 length polymorphism and increased odds of PTD, and polymorphisms in the IL1-RN gene and the MMP-9 gene and increased odds of SPT PTD. Among African-Americans the TNFR2 G allele was associated with increased odds of SPT PTD. In both racial groups none of the maternal polymorphisms were significantly associated with only MI PTD.

Results for the child polymorphisms are presented in Table IV. In non-Hispanic whites statistically significant findings included the IL1-RN allele 2 length polymorphism and increased odds of both PTD and SPT PTD and an inverse association between TNFR2 allele G and odds of MI PTD. A statistically significant inverse relation was found for TNF-α allele A and PTD in African Americans.

Maternal and child polymorphism interactions are presented in Table V. Among non-Hispanic white mother/child pairs, the odds of PTD was significantly increased when both

mother and child had the IL1-RN 2 allele (odds ratio = 2.3; 95% confidence interval 1.3, 3.8, significant additive interaction), but not when this variant allele was present only in the mother or child. Among African-American mother/child pairs, the odds of PTD was increased when both mother and child carried the TNFR2 G allele (odds ratio 1.6; 95% confidence interval 0.8, 3.4, significant multiplicative interaction) but not when this allele was present only in the mother or child.

Discussion

We studied ten polymorphisms in nine genes regulating innate immunity in the mother and her child. Eight of the ten polymorphism have been linked to PTD in reports of maternal genotype, but these associations have been inconsistent [12,14,15,33-35]. Several of our findings paralleled results from previous studies. We were able to replicate associations between SPT PTD and both maternal and child IL1-RN allele 2 length polymorphism in the non-Hispanic whites [13,36]. The TNF-α A allele was protective for PTD among African-American children [37]. In addition, we identified two new positive associations between spontaneous PTD and a maternal polymorphism, one with MMP-9 in the non-Hispanic whites and the other with TNFR2 in African-Americans. We also showed that maternal IL1-RN allele 2 confers a risk of PTD in the non-Hispanic white mothers only if her child also inherits this allele. The same phenomenon was observed for the TNFR2 polymorphism in African-Americans

In the initial design of our study, we hypothesized that the effects of polymorphisms might differ for SPT PTD versus MI PTD, though inflammatory responses could be relevant to both. Most prior genetic studies of preterm birth have excluded MI PTD. Our statistically significant findings were found primarily in relation to SPT PTD, however, in many analyses the number of MI PTD in a given stratum were too few to allow firm conclusions. Larger studies will be needed to explore genetic risk factors across clinical subsets of PTD.

Based on birth certificate data, the POUCH Study cohort participants were representative of the communities from which they were sampled. Although only 68% of the eligible sub-cohort women participated in this follow up study, there were no differences in maternal demographic characteristics or allelic frequencies between women whose children were included in the genetic analysis and women in the entire sub-cohort. Only 1% (19) of the children were deceased at the time of DNA collection. It is therefore unlikely that significant bias was introduced due to sampling or genetic characteristics associated with survival.

This and other studies demonstrate that the inflammation- related polymorphisms associated with PTD may differ across s race/ethnicity groups. These variations suggest the importance of gene-gene and gene-environment interactions in inflammatory pathway affecting PTD risk. Genetic association studies focusing on PTD should therefore account for ethnic variation. In this study we stratified our study population according to race/ethnicity self-reported by the mother. In our data, most maternal allele frequencies were significantly different by race. Paternal race was reported to be the same as the mother's in 96% of non-Hispanic-white women and in 89% of African-American women. We relied on mother's report to define her and her partner's race and did not identify ancestry informative markers

that are often used to reduce bias due to population stratification. At least one study suggests that this the bias may be minimal in US Caucasian populations [38].

The statistically significant associations between PTD and genetic variation observed in this study would have been lost had we corrected for multiple comparisons. It is therefore possible that some of the associations reported here may be spurious. It is, however, notable that the association with SPT PTD and ILRN has been independently confirmed by other studies [12]. Our findings related to MMP-9 1562 and TNFR2 198 polymorphisms are novel and await future confirmation.

This study was adequately powered (β=80%) to detect an odds ratio of 2.0 associated with an adverse outcome and presence of a minor allele with a prevalence of 15%. However, it lacked the power to analyze interactions between multiple genetic markers and haplotypes. We also lacked statistical power to subdivide PTD further by gestational weeks. Therefore associations tightly linked to gestational age-related PTD phenotypes may have been missed. It is also possible that we failed to identify associations for the more rare variants. Notably we had limited power to test for interactions for some gene-gene combinations and therefore our negative findings could be the result of type II error.

Replication is an important part of the process to understand the strength and consistency of relations among specific genes and PTD across a variety of methodologic approaches. As part of this process, both positive and negative findings are informative. To date, genetic studies of PTD have struggled with reproducibility. Differences across study populations in genetic background and environmental exposures may be important contributors to the inconsistent results. Our study approached the question by trying to replicate findings from previous studies, by selecting candidate functional polymorphisms and by specifically examining maternal and child genotype combinations. In a recent review, the challenges of non-replication in gene association studies are highlighted and include issues of polymorphism selection, bias due to population stratification, low power, and reporting bias [39].

A recent large-scale candidate gene study in a Chilean population included polymorphisms in both maternal and child DNA and used several different analytic techniques (i.e. single locus tests, haplotype tests, multi-locus analysis, pathway analysis) to explore genetic associations in relation to PTD [10]. Across multiple analytic techniques, their findings were consistent with the hypothesis that genetic variations along the inflammation pathway in both mother and fetus are important risk factors for PTD. In addition, they identified a significant three-way interaction between a maternal polymorphism (collagen type IV alpha 4) and two fetal polymorphisms (collagen type IV alpha 4 and fibroblast growth factor 1). The importance of the specific polymorphisms identified by their work may not be directly comparable to the findings in our study due to differences in ethnicity and environmental exposures across our study populations.

Our findings, although preliminary, in combination with others suggest that genetic models examining the risk of PTD should consider interactions between maternal and child

genotypes. These models will be potentially more illuminating when gene-environment interactions are also incorporated.

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Table I

Maternal demographic characteristics and pregnancy outcomes (Weighted percentages reflect cohort prevalence)

	Non-Hispanic White (N=487)			African American (N=288)		
Maternal Characteristics	**N**	**Unweighted %**	**Weighted %**	**N**	**Unweighted %**	**Weighted %**
Maternal Age (years)						
<20	45	9	9	67	23	24
20-29	258	53	53	178	62	63
≥30	184	38	38	43	15	14
Maternal Education (years)						
<12	47	10	9	94	31	34
12	124	25	25	85	29	30
>12	316	65	66	109	38	36
Medicaid Insured*						
No	334	69	70	61	21	20
Yes	152	31	30	227	79	80
Parity*						
0	210	43	40	115	40	41
≥1	276	57	60	173	60	59
Pregnancy Outcome						
Term delivery	356	73	90	239	83	86
Medically indicated preterm delivery	38	8	3	11	4	3
Spontaneous preterm delivery	93	19	7	38	13	11

* Data on Medicaid status and parity is missing for 1 non-Hispanic White woman

Table II

Inflammation Gene Polymorphisms and race specific minor allele frequencies unweighted for sub-cohort sampling scheme

		Maternal Minor Allele Frequency		Child Minor Allele Frequency	
Gene/Polymorphism	**Description**	**Non-Hispanic White N=487***	**African American N=288***	**Non-Hispanic White N=487***	**African American N=288***
CD14 -C159T (rs# 73271540)	Binds LPS and regulates pro-inflammatory immunity.	Term = .49 Preterm =.51	Term = .38 Preterm =.40	Term =.47 Preterm =.44	Term = .36 Preterm =.40
IL-1β C3954T (rs# 1143634)	Pro-inflammatory function. Can stimulate the production of prostaglandins.	Term =.21 Preterm =.23	Term = .14 Preterm =.14	Term =.22 Preterm =.23	Term =.12 Preterm =.14
IL-1RN allele 2 Variable number of tandem repeats in intron 2	Competitive inhibitor of IL-1.	Term = .24 Preterm =.33	Term=.09 Preterm=.04	Term =.24 Preterm =.32	Term=.10 Preterm=.11
MBL G54A (rs# 1800450)	Acute phase serum protein involved in antimicrobial innate immunity and activation of the lectin complement pathway.	Term= .11 Preterm =.12	Term=.04 Preterm=.06	Term= .11 Preterm =.10	Term=.04 Preterm=.04
MBL G57A (rs# 1800451)	Acute phase serum protein involved in antimicrobial innate immunity and activation of the lectin complement pathway.	Term = .03 Preterm =.04	Term=.19 Preterm=.21	Term = .04 Preterm =.04	Term=.21 Preterm=.24
MMP-9 -C1562T (rs# 73622645)	Enzyme that degrades collagen type IV, elastin, and fibronectin.	Term = .13 Preterm = .19	Term=.12 Preterm=.13	Term =.13 Preterm = .11	Term=.14 Preterm=.16
TLR4 A896G (rs# 4986790)	Membrane-bound pattern recognition receptor for LPS binding protein with pro-inflammatory function.	Term = .05 Preterm =.07	Term=.06 Preterm=.02	Term = .06 Preterm =.08	Term=.09 Preterm=.04
TNF-α -G308A (rs# 1800629)	Associated with pro-inflammatory immunity and programmed cell death.	Term= .18 Preterm =.16	Term=.16 Preterm=.07	Term= .18 Preterm = .14	Term=.18 Preterm=.08
TNFR2 T198G (rs# 72863489)	Binding is associated with activation of pro-inflammatory immunity.	Term =.25 Preterm =.26	Term=.19 Preterm=.30	Term = .23 Preterm =.24	Term=.18 Preterm=.18
TNFRSF6 -A670G (rs# 978522)	Binding results in activation of apoptotic signal transmission.	Term = .48 Preterm =.42	Term=.72 Preterm=.70	Term = .48 Preterm =.46	Term=.71 Preterm=.63

LPS = lipopolysacharride, IL = Interleukin

*Polymorphism data missing on 0-12 non-Hispanic white mothers, 0-3 African-American mothers, 0-6 non-Hispanic white children, and 0-6 African-American children.

Table III
Maternal gene polymorphisms and preterm delivery

			Preterm Subtype	
		Preterm Overall	Medically Indicated	Spontaneous
Gene	**Ethnicity**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
CD14 -C159T (rs# 73271540)	Non-Hispanic white	0.9 (0.5, 1.4)	0.9 (0.4, 1.9)	0.9 (0.5, 1.5)
	African American	1.5 (0.8, 3.0)	2.0 (0.5, 8.4)	1.4 (0.6, 3.0)
IL-1β C3954T (rs# 1143634)	Non-Hispanic white	1.0 (0.6, 1.5)	0.7 (0.3, 1.4)	1.2 (0.7, 1.9)
	African American	0.6 (0.2, 1.3)	NE	0.8 (0.3, 1.9)
IL-1RN allele 2, Intron 2 repeats	Non-Hispanic white	1.9 (1.2, 2.9)*	1.6 (0.8, 3.2)	2.0 (1.2, 3.3)*
	African American	0.4 (0.1, 1.3)	0.8 (0.2, 4.1)	0.3 (0.1, 1.4)
MBL G54A (rs# 1800450)	Non-Hispanic white	1.3 (0.8, 2.2)	0.8 (0.3, 2.0)	1.5 (0.9, 2.7)
	African American	1.3 (0.4, 3.8)	NE	1.8 (0.6, 5.2)
MBL G57A (rs# 1800451)	Non-Hispanic white	1.3 (0.6, 3.0)	1.6 (0.4, 5.6)	1.3 (0.5, 3.2)
	African American	1.2 (0.6, 2.2)	1.1 (0.3, 4.0)	1.2 (0.6, 2.4)
MMP-9 -C1562T (rs# 73622645)	Non-Hispanic white	1.5 (0.9, 2.3)	1.1 (0.5, 2.3)	1.7 (1.0, 2.8)*
	African American	1.2 (0.6, 2.5)	1.1 (0.3, 4.5)	1.2 (0.5, 2.8)
TLR4 A896G (rs# 4986790)	Non-Hispanic white	1.4 (0.8, 2.7)	1.9 (0.7, 5.0)	1.3 (0.6, 2.6)
	African American	0.4 (0.1, 1.8)	0.9 (0.1, 7.5)	0.3 (0.0, 2.0)
TNF-α -G308A (rs# 1800629)	Non-Hispanic white	0.9 (0.6, 1.4)	1.2 (0.6, 2.6)	0.8 (0.4, 1.3)
	African American	0.4 (0.2, 1.0)	0.5 (0.1, 2.6)	0.4 (0.1, 1.1)
TNFR2 T198G (rs# 72863489)	Non-Hispanic white	1.1 (0.7, 1.7)	0.7 (0.3, 1.4)	1.3 (0.8, 2.1)
	African American	1.6 (0.9, 3.1)	0.4 (0.1, 2.1)	2.3 (1.1, 4.6)*
TNFRSF6 -A670G (rs# 978522)	Non-Hispanic white	0.7 (0.4, 1.1)	0.8 (0.4, 1.6)	0.7 (0.4, 1.1)
	African American	0.7 (0.2, 2.3)	NE	0.5 (0.2, 1.8)

CI = Confidence Interval, NE = Not Estimable, OR= Odds Ratio

* p<.05

Table IV
Child gene polymorphisms and preterm delivery

			Preterm Subtype	
		Preterm Overall	Medically Indicated	Spontaneous
Gene	**Ethnicity**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
CD14 -C159T (rs# 73271540)	Non-Hispanic white	0.8 (0.5, 1.2)	0.9 (0.4, 1.8)	0.7 (0.4, 1.2)
	African American	1.0 (0.5, 2.0)	0.8 (0.2, 2.7)	1.1 (0.5, 2.3)
IL-1β C3954T (rs# 1143634)	Non-Hispanic white	1.0 (0.6, 1.5)	0.8 (0.4, 1.6)	1.1 (0.7, 1.8)
	African American	1.2 (0.6, 2.5)	0.2 (0.0, 1.5)	1.7 (0.7, 3.6)
IL-1RN allele 2, Intron 2 repeats	Non-Hispanic white	1.6 (1.1, 2.5)*	1.5 (0.7, 3.0)	1.7 (1.0, 2.8)*
	African American	1.2 (0.6, 2.7)	1.8 (0.4, 7.4)	1.1 (0.4, 2.7)
MBL G54A (rs# 1800450)	Non-Hispanic white	1.0 (0.6, 1.6)	1.3 (0.6, 2.9)	0.8 (0.5, 1.5)
	African American	1.1 (0.3, 3.5)	NE	1.5 (0.4, 4.9)
MBL G57A (rs# 1800451)	Non-Hispanic white	1.1 (0.5, 2.3)	1.2 (0.3, 4.2)	1.0 (0.4, 2.5)
	African American	1.5 (0.8, 2.9)	1.5 (0.4, 5.1)	1.5 (0.7, 3.2)
MMP-9 -C1562T (rs# 73622645)	Non-Hispanic white	0.9 (0.6, 1.5)	0.8 (0.3, 1.8)	1.0 (0.6, 1.7)
	African American	1.0 (0.5, 2.1)	3.0 (0.9, 11.0)	0.7 (0.3, 1.6)
TLR4 A896G (rs# 4986790)	Non-Hispanic white	1.4 (0.8, 2.5)	1.2 (0.4, 3.3)	1.5 (0.8, 2.8)
	African American	0.5 (0.2, 1.4)	1.1 (0.2, 5.5)	0.3 (0.1, 1.3)
TNF-α -G308Am (rs# 1800629)	Non-Hispanic white	0.7 (0.5, 1.2)	0.5 (0.2, 1.2)	0.8 (0.5, 1.4)
	African American	0.4 (0.2, 1.0)*	0.5 (0.1, 2.4)	0.4 (0.2, 1.1)
TNFR2 T198G (rs# 72863489)	Non-Hispanic white	1.1 (0.7, 1.6)	0.3 (0.1, 0.7)*	1.6 (1.0, 2.2)
	African American	1.0 (0.5, 1.9)	0.2 (<.1, 1.8)	1.3 (0.6, 2.7)
TNFRSF6 -A670G (rs# 978522)	Non-Hispanic white	1.0 (0.6, 1.6)	1.0 (0.5, 2.2)	1.0 (0.6, 1.7)
	African American	0.7 (0.2, 1.7)	0.4 (0.1, 2.2)	0.8 (0.2, 2.4)

CI = Confidence Interval, NE = Not Estimable, OR = Odds Ratio

* p<.05

Table V

Combinations of Maternal-Child Genotype and odds of Preterm Delivery

	Maternal & Child Minor Allele Carriage	Total Number	N Preterm	OR (95% C I)	Multiplicative Interaction p-value*	Significant Additive Interaction?†
MMP9 (rs# 73622645) **non-Hişpanic white**	Mom No Child No	295	81	1.0	0.06	No
	Mom No Child Yes	46	6	0.4 (0.2, 1.0)		
	Mom Yes Child No	68	21	1.2 (0.6, 2.1)		
	Mom Yes Child Yes	71	23	1.5 (0.8, 2.6)		
IL1RN, Intron 2 repeats **non-Hispanic white**	Mom No Child No	175	39	1.0	0.13	Yes
	Mom No Child Yes	68	14	0.9 (0.5, 1.9)		
	Mom Yes Child No	75	19	1.1 (0.6, 2.2)		
	Mom Yes Child Yes	130	49	2.3 (1.3, 3.8)		
TNF-α (rs# 1800629) **African American**	Mom No Child No	169	39	1.0	0.08	No
	Mom No Child Yes	37	2	0.2 (0.0, 1.0)		
	Mom Yes Child No	32	2	0.2 (0.1, 1.3)		
	Mom Yes Child Yes	48	5	0.4 (0.2, 1.2)		
TNFR2 (rs# 72863489) **African American**	Mom No Child No	144	26	1.0	0.01	No
	Mom No Child Yes	35	1	0.1 (0.0, 1.6)		
	Mom Yes Child No	46	7	0.9 (0.3, 2.2)		
	Mom Yes Child Yes	60	15	1.6 (0.8, 3.4)		

CI = Confidence Interval, OR = Odds Ratio, NE- Not estimable

*Multiplicative interaction calculated using weighted logistic regression and incorporated weights from sampling into the cohort and sub-cohort.

†Additive interaction calculated using relative excess risk method.